Set Name side by side	Query	Hit Count	Set Name result set
	DB=USPT,PGPB,JPAB,EPAB,DWPI,TDBD; THES=ASSIGNEE; PLUR=YES; OP=AND		
L11	L9 not L10	47	L11
L10	L9 and (ubiquitin or (signal adj sequence))	30	L10
L9	L7 and (mucosal or systemic)	77	L9
L8	L7 and ((expression adj library) adj immunization)	1	L8
L7	L5 and (vaccine)	132	L7
L6	L5 and (macroaggregated or aggregated)	30	L6
L5	L3 and (antigen)	427	L5
L4	L3 and (genetic adj vaccine)	1	L4
L3	L2 same (complexes or conjugate or aggregate)	542	L3
L2	(vector or DNA or plasmid) same (polylysine or PEI or polyethyleneimine or (polycationic adj polymer)) same (albumin or transferrin or ligand or protein)	921	L2
L1	Orson-frank-M$.in.	0	L1

END OF SEARCH HISTORY

```
### Status: Path 1 of [Dialog Information Services via Modem]

### Status: Initializing TCP/IP using (UseTelnetProto 1 ServiceID pto-dialog)
Trying 31060000009999...Open

DIALOG INFORMATION SERVICES
PLEASE LOGON:
 ******** HHHHHHHH SSSSSSSS?
### Status: Signing onto Dialog
 ********
ENTER PASSWORD:
 ******** HHHHHHHH SSSSSSSS? ********
Welcome to DIALOG
### Status: Connected


Dialog level 02.09.15D

Last logoff:  16oct02 12:01:05
Logon file001 17oct02 12:33:39
           *** ANNOUNCEMENT ***
                    ***
--The following files from Cambridge Scientific Abstracts (CSA)
are no longer available: 14, 28, 32, 33, 36, 37, 41, 44, 56, 61,
76, 77, 108, 117, 232, 238, 269, 293, 335. Please enter HELP CSA
plus the file number to identify alternative sources of information.
Example: HELP CSA14.
                    ***
--File 515 D&B Dun's Electronic Business Directory is now online
completely updated and redesigned. For details, see HELP NEWS 515.
                    ***
--File 990 - NewsRoom now contains May 2002 to present records.
File 993 - NewsRoom archive contains 2002 records from January 2002-
April 2002. To search all 2002 records, BEGIN 990,993 or B NEWS2002.
                    ***
--Alerts have been enhanced to allow a single Alert profile to be
stored and run against multiple files. Duplicate removal is available
across files and for up to 12 months. The Alert may be run according
to the file's update frequency or according to a custom
calendar-based schedule. There are no additional prices for these
enhanced features. See HELP ALERT for more information.
                    ***
--U.S. Patents Fulltext (File 654) has been redesigned with
new search and display features.  See HELP NEWS 654 for
information.
                    ***
--Connect Time joins DialUnits as pricing options on Dialog.
See HELP CONNECT for information.
                    ***
--CLAIMS/US Patents (Files 340,341, 942) have been enhanced
with both application and grant publication level in a
single record. See HELP NEWS 340 for information.
                    ***
--SourceOne patents are now delivered to your email inbox
as PDF replacing TIFF delivery. See HELP SOURCE1 for more
information.
                    ***
--Important news for public and academic
libraries.  See HELP LIBRARY for more information.
                    ***
--Important Notice to Freelance Authors--
See HELP FREELANCE for more information
                    ***
For information about the access to file 43 please see Help News43.
***
```

NEW FILES RELEASED
***Dialog NewsRoom - Current 3-4 months (File 990)
***Dialog NewsRoom - 2002 Archive (File 993)
***Dialog NewsRoom - 2001 Archive (File 994)
***Dialog NewsRoom - 2000 Archive (File 995)
***TRADEMARKSCAN-Finland (File 679)
***TRADEMARKSCAN-Norway (File 678)
***TRADEMARKSCAN-Sweden (File 675)
***
UPDATING RESUMED
***Delphes European Business (File 481)
***
RELOADED
***D&B Dun's Electronic Business Directory (File 515)
***U.S. Patents Fulltext 1976-current (File 654)
***Population Demographics (File 581)
***Kompass Western Europe (File 590)
***D&B - Dun's Market Identifiers (File 516)

REMOVED
***Chicago Tribune (File 632)
***Fort Lauderdale Sun Sentinel (File 497)
***The Orlando Sentinel (File 705)
***Newport News Daily Press (File 747)
***U.S. Patents Fulltext 1980-1989 (File 653)
***Washington Post (File 146)
***Books in Print (File 470)
***Court Filings (File 793)
***Publishers, Distributors & Wholesalers of the U.S. (File 450)
***State Tax Today (File 791)
***Tax Notes Today (File 790)
***Worldwide Tax Daily (File 792)
***ISMEC: Mechanical Engineering Abstracts (File 14)
***Oceanic Abstracts (File 28)
***METADEX: Metals Science (File 32)
***Aluminium Industry Abstracts (File 33)
***Linguistics and Language Behavior Abstracts (File 36)
***Sociological Abstracts (File 37)
***Pollution Abstracts (File 41)
***Aquatic Sciences and Fisheries Abstracts (File 44)
***ARTbibliographies Modern (File 56)
***LISA (Library & Information Science Abstracts) (File 61)
***Life Sciences Collection (File 76)
***Conference Papers Index (File 77)
***Aerospace Database (File 108)
***Water Resources Abstracts (File 117)
***Applied Social Sciences Index and Abstracts (File 232)
***Abstracts in New Technologies and Engineering (File 238)
***Materials Business File (File 269)
***Engineered Materials Abstracts (File 293)
***Ceramic Abstracts (File 335)

New document supplier
IMED has been changed to INFOTRIE (see HELP OINFOTRI)

>>> Enter BEGIN HOMEBASE for Dialog Announcements <<<
>>> of new databases, price changes, etc. <<<
****
KWIC is set to 50.
HILIGHT set on as '*'
*** ***

File 1:ERIC 1966-2002/Oct 03

```
      Set  Items  Description
      ---  -----  -----------
Cost is in DialUnits
?b 155, 159, 5, 55
       17oct02 12:33:56 User259876 Session D419.1
            $0.82    0.233 DialUnits File1
     $0.82  Estimated cost File1
     $0.05  TELNET
     $0.87  Estimated cost this search
     $0.87  Estimated total session cost   0.233 DialUnits


SYSTEM:OS  - DIALOG OneSearch
  File 155:MEDLINE(R)  1966-2002/Oct W2
*File 155: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.
  File 159:Cancerlit  1975-2002/Sep
         (c) format only 2002 Dialog Corporation
  File   5:Biosis Previews(R)  1969-2002/Oct W2
         (c) 2002 BIOSIS
*File   5: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.
  File  55:Biosis Previews(R)  1993-2002/Oct W2
         (c) 2002 BIOSIS
*File  55: Alert feature enhanced for multiple files, duplicates
removal, customized scheduling. See HELP ALERT.

      Set  Items  Description
      ---  -----  -----------
?s (protein-polyeethylenimine-plasmid) or (protein/polyethylenimine/plasmid)
>>>Invalid term in limit list: POLYETHYLENIMINE
?s (protein/polyethylenimine/plasmid)
>>>Invalid term in limit list: POLYETHYLENIMINE
?s (protein/polyethyleneimine/plasmid)
>>>Invalid term in limit list: POLYETHYLENEIMINE
?s (DNA/transferrin-PEI)
>>>Invalid term in limit list: TRANSFERRIN
?s (plasmid or DNA) (s) (PEI or polyethyleneimine) (s) (albumin or transferrin or prote
in)
         159257  PLASMID
        1938791  DNA
           3035  PEI
           1857  POLYETHYLENEIMINE
         220248  ALBUMIN
          53121  TRANSFERRIN
        3300634  PROTEIN
      S1     195  (PLASMID OR DNA) (S) (PEI OR POLYETHYLENEIMINE) (S)
                  (ALBUMIN OR TRANSFERRIN OR PROTEIN)
?s s1 and (conjugates or complexes)
           195  S1
         44667  CONJUGATES
        295637  COMPLEXES
      S2      89  S1 AND (CONJUGATES OR COMPLEXES)
?s s2 and (antigen)
            89  S2
        828966  ANTIGEN
      S3      10  S2 AND (ANTIGEN)
?rd
...completed examining records
      S4       3  RD (unique items)
?t s4/3,k/all

 4/3,K/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11164817   21214798   PMID: 11313828
```

MHC class II presentation of endogenously expressed antigens by transfected dendritic cells.

Diebold S S; Cotten M; Koch N; Zenke M
Max-Delbruck-Center for Molecular Medicine, MDC, Berlin, Germany.

Gene therapy (England) Mar 2001, 8 (6) p487-93, ISSN 0969-7128
Journal Code: 9421525
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

... I and class II molecules in association with costimulatory molecules, and efficiently activate both cytotoxic T cells and T helper cells. Gene modified DC expressing *antigen* encoding cDNA represent a particularly attractive approach for the immunotherapy of disease. We previously described a gene delivery system for DC based on receptor-mediated endocytosis of ligand/polyethylenimine (*PEI*) *DNA* transfer *complexes* that target cell surface receptors which are abundantly expressed on DC. Employing this gene delivery system, DC were generated that express chicken ovalbumin (OVA) cDNA as a model *antigen* and introduce *antigen* into the MHC class I presentation pathway. We demonstrate here that modification of OVA cDNA as *transferrin* receptor (TfR) or invariant chain (Ii) fusions effectively generate MHC class II specific immune responses in addition to MHC class I responses. TfR-OVA contains the membrane anchoring region of *transferrin* receptor and represents a membrane-bound form of OVA for access to the MHC class II compartment. Ii-OVA fusions directly target the MHC class II processing pathway. Thus, modification of *antigen* encoding cDNA represents a convenient and effective means to direct antigens to MHC class II presentation and thus to generate T cell help.

4/3,K/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10316864 99315848 PMID: 10383411

Mannose polyethylenimine *conjugates* for targeted DNA delivery into dendritic cells.

Diebold S S; Kursa M; Wagner E; Cotten M; Zenke M
Max-Delbruck-Center for Molecular Medicine, Robert-Rossle-Str. 10, D-13092 Berlin, Germany.

Journal of biological chemistry (UNITED STATES) Jul 2 1999, 274 (27) p19087-94, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Mannose polyethylenimine *conjugates* for targeted DNA delivery into dendritic cells.

... entry sites for gene delivery into cells by receptor-mediated endocytosis. Here we have taken advantage of the mannose receptor that is highly expressed on *antigen*-presenting dendritic cells for targeted gene transfer by employing mannosylpolyethylenimine (ManPEI) *conjugates* . Several ManPEI *conjugates* were synthesized and used for formation of ManPEI/*DNA* transfection *complexes*. *Conjugates* differed in the linker between mannose and polyethylenimine (*PEI*) and in the size of the *PEI* moiety. We demonstrate that ManPEI transfection is effective in delivering *DNA* into mannose receptor-expressing cells. Uptake of ManPEI/*DNA* *complexes* is receptor-specific, since *DNA* delivery can be competed with mannosylated *albumin*. Additionally, incorporation of adenovirus particles into transfection *complexes* effectively enhances transgene expression. This is particularly important for primary immunocompetent dendritic cells. It is demonstrated here that dendritic cells transfected with ManPEI/*DNA* *complexes* containing adenovirus particles are effective in activating T cells of T cell receptor transgenic mice in an *antigen*-specific fashion.

4/3,K/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10234377 99224851 PMID: 10210145
Efficient gene delivery into human dendritic cells by adenovirus polyethylenimine and mannose polyethylenimine transfection.
Diebold S S; Lehrmann H; Kursa M; Wagner E; Cotten M; Zenke M
Max-Delbruck-Center for Molecular Medicine, Berlin, Germany.
Human gene therapy (UNITED STATES) Mar 20 1999, 10 (5) p775-86,
ISSN 1043-0342 Journal Code: 9008950
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Gene-modified human dendritic cells (DCs) were generated by transfection with adenovirus polyethylenimine *DNA* (Ad/*PEI*/*DNA*) and mannose polyethylenimine *DNA* (ManPEI/*DNA*) *complexes*. Ad/*PEI*/*DNA* *complexes* have *plasmid* *DNA* bound to adenovirus particles by *PEI* and deliver *DNA* into cells via the adenovirus infection route. Such transfection *complexes* yield high transduction levels and sustained expression of luciferase and green fluorescent *protein* reporter genes and were almost as effective as recombinant adenovirus vectors. ManPEI/*DNA* *complexes* rely on uptake by receptor-mediated endocytosis via mannose receptor, which is highly expressed on DCs. While gene delivery by ManPEI/*DNA* *complexes* was less efficient than by Ad/*PEI* transfection, incorporation of adenovirus particles in ManPEI/*DNA* transfection *complexes* further enhanced transduction efficiencies and transgene expression. We also demonstrate that Ad/*PEI*-transfected DCs are competent in stimulating T cell proliferation in allogeneic and autologous mixed lymphocyte reactions, and in activating T cells from T cell receptor (TCR)-transgenic mice in an *antigen*-specific manner. Thus, the present study establishes the following relative order of transduction efficiencies of viral and nonviral gene delivery systems for primary human DCs: recombinant adenovirus > Ad/*PEI* = Ad/ManPEI > ManPEI > *PEI*. Ad/*PEI* and ManPEI transfection modes represent particularly versatile transduction systems for DCs, with ManPEI being built up exclusively of synthetic compounds.
?
?ds

Set Items Description
S1 195 (PLASMID OR DNA) (S) (PEI OR POLYETHYLENEIMINE) (S) (ALBUMIN OR TRANSFERRIN OR PROTEIN)
S2 89 S1 AND (CONJUGATES OR COMPLEXES)
S3 10 S2 AND (ANTIGEN)
S4 3 RD (unique items)
?s s2 and (aggregated or aggregates)
89 S2
31111 AGGREGATED
57106 AGGREGATES
S5 16 S2 AND (AGGREGATED OR AGGREGATES)
?rd
...completed examining records
S6 6 RD (unique items)
?t s6/3,k/all

6/3,K/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12860508 21616843 PMID: 11741272
DNA/polyethylenimine transfection particles: influence of ligands, polymer size, and PEGylation on internalization and gene expression.
Ogris M; Steinlein P; Carotta S; Brunner S; Wagner E
Institute of Biochemistry, University of Vienna, Vienna, Austria.

manfred.ogris@cup.uni-muenchen.de
AAPS PharmSci (United States) 2001, 3 (3) pE21, ISSN 1522-1059
Journal Code: 100897065
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Receptor-binding ligands have been incorporated into *DNA* /polyethylenimine (*PEI*) *complexes* to enhance cell binding and cellular internalization. This study characterizes receptor-mediated uptake of *DNA* /*PEI* *complexes* on a cellular basis. A novel assay based on flow cytometry was applied, discriminating between total cell-associated and extracellularly bound *DNA* *complexes* . Receptor-mediated uptake of ligand-containing *DNA*/*PEI* (molecular weight, 800 kd) *complexes* was found to occur quickly (within 1 hour), whereas unspecific uptake through adsorptive endocytosis is less efficient or requires extended periods to reach the same degree of internalization. Rapid, receptor-mediated internalization requires a small complex size; however, large, *aggregated* *complexes* show higher gene expression. Using *PEI* 25 kd conjugated to large proteins such as *transferrin* or antibodies, improper condensation with *DNA* leads to suboptimal uptake and gene expression, whereas partial replacement of ligand-*PEI* with unconjugated *PEI* increases both uptake and transfection. In contrast, the 8 kd *protein* epidermal growth factor conjugated to *PEI* 25 kd properly condenses *DNA* and mediates specific uptake into human adenocarcinoma (KB) cells. Modification of the complex surface with appropriate amounts of poly(ethylene glycol) (PEG) does not block ligand-mediated internalization. A higher degree of PEGylation reduces the internalization of *transferrin* or antibody-containing *complexes* to a level similar to that of ligand-free *complexes*. In contrast, epidermal growth factor "mediated uptake is less effected by excessive PEGylation.

6/3,K/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

12798265 21420347 PMID: 11529666
Different behavior of branched and linear polyethylenimine for gene delivery in vitro and in vivo.
Wightman L; Kircheis R; Rossler V; Carotta S; Ruzicka R; Kursa M; Wagner E
Department of Cancer Vaccines & Gene Therpy, Boehringer Ingelheim Austria GmbH, Vienna. lionel.wightman@vie.boehringer-ingelheim.com
journal of gene medicine (England) Jul-Aug 2001, 3 (4) p362-72, ISSN 1099-498X Journal Code: 9815764
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

... Understanding how non-viral vectors initiate gene expression could lead to the development of new future vectors with enhanced efficacy. METHODS: Linear or branched polyethylenimine (*PEI*)/*DNA* *complexes* were generated in varying salt conditions and their transfection efficiencies were compared in vitro and in vivo using reporter genes, luciferase and green fluorescent *protein*, and rhodamine labeled *DNA* (pGeneGrip). RESULTS: The transfection efficiency of linear PEI22/*DNA* in vitro was generally greater than that of branched *PEI*/*DNA* when *complexes* were generated in salt containing buffer. However, *PEI* *complexes* generated under salt-free conditions generally had low transfection activity in vitro. In contrast, PEI22/*DNA* salt-free *complexes* were highly active in vivo. Branched *PEI*/*DNA* and salt containing PEI22/*DNA* *complexes* were generally 10-100-fold less active than the salt-free PEI22/*DNA* *complexes*. Salt-free PEI22/*DNA* *complexes* were small, but subsequently grew into *aggregates* when salt was added. In contrast, PEI25/*DNA*

complexes remained small even after salt was added under the same conditions. Furthermore, PEI22/pGeneGrips *complexes* formed large *aggregates* associated with the cell membrane, cytoplasm and nucleus, while branched *PEI* *complexes* remained as small distinct particles associated with the cell membrane or in the cytoplasm. CONCLUSIONS: Branched and linear *PEI*/*DNA* *complexes* differ in their ability to transfect cells. The greater efficiency of linear *PEI* might be due to an inherent kinetic instability under salt conditions. Understanding how to employ this kinetic instability of linear *PEI* could help in designing future vectors with greater flexibility and transfection efficiency in vivo.

6/3,K/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10966052 20534468 PMID: 11083497
Biodistribution and transgene expression with nonviral cationic vector/DNA *complexes* in the lungs.
Bragonzi A; Dina G; Villa A; Calori G; Biffi A; Bordignon C; Assael B M; Conese M
Institute for Experimental Treatment of Cystic Fibrosis, San Raffaele Scientific Institute, Milano, Italy.
Gene therapy (ENGLAND) Oct 2000, 7 (20) p1753-60, ISSN 0969-7128 Journal Code: 9421525
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Biodistribution and transgene expression with nonviral cationic vector/DNA *complexes* in the lungs.
Biodistribution of nonviral cationic vector/*DNA* *complexes* was studied after systemic or intratracheal administration to the lungs and correlated with transgene expression. Intravenous injection in C57Bl/6 mice gave maximal and significant luciferase expression in the lungs with the cationic polymer *PEI* 22K/*DNA* *complexes* at the highest ratios of positive/negative charges versus *DNA* alone. While DOTAP/*DNA* *complexes* with high charge ratio determined lower but still significant luciferase activity versus uncomplexed *DNA*, GL-67A and *PEI* 25K mediated negligible luciferase expression. Labelled *PEI* 22K and DOTAP *complexes* were evenly distributed in the alveolar region, where GFP expression was revealed, while *PEI* 25K and GL-67A *complexes* were not detected, suggesting a different interaction of these *complexes* with the plasma membrane of endothelial cells. Following an intratracheal injection, the highest and significant levels of transfection were obtained with slightly positive *PEI* *complexes* as compared with *DNA* alone, whereas cationic lipid-based vectors, DOTAP and GL-67A, gave not significant luciferase activity. Both types of polyplexes gave similar levels of lung luciferase expression by targeting different airway cell populations. *PEI* 25K *complexes* determined high levels of GFP in the bronchial cells, confirming confocal data on fluorescent *complexes* internalization. *PEI* 22K *complexes* gave mainly high GFP signal in the distal tract of the bronchial tree, where tagged *complexes* were recovered. Fluorescent lipid *complexes* were found in *aggregates* in the lumen of bronchi totally (DOTAP) or partially (GL-67A) co-localizing with surfactant *protein* A. Results indicated that cationic polymers could overcome the surfactant barrier which inhibited airway cell transfection mediated by cationic lipids.

6/3,K/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10754932 20302782 PMID: 10843685
Genetic immunization with lung-targeting macroaggregated

polyethyleneimine-albumin *conjugates* elicits combined systemic and mucosal immune responses.

Orson F M; Kinsey B M; Hua P J; Bhogal B S; Densmore C L; Barry M A

Veterans Affairs Medical Center, Baylor College of Medicine, Houston, TX 77030, USA. forson@bcm.tmc.edu

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Jun 15 2000, 164 (12) p6313-21, ISSN 0022-1767 Journal Code: 2985117R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Genetic immunization with lung-targeting macroaggregated polyethyleneimine-albumin *conjugates* elicits combined systemic and mucosal immune responses.

Genetic immunization is a novel form of vaccination in which transgenes are delivered into the host to produce the foreign *protein* within host cells. Although systemic immune responses have been relatively easy to induce by genetic immunization, the induction of regional and mucosal immunity has often been more challenging. To address the problem of eliciting mucosal immunity in the lung, we utilized macroaggregated *albumin* to target *plasmid* *DNA* to the lung. Macroaggregated *albumin* is trapped in the lung after i. v. injection, and it is routinely used in radiolabeled form as an imaging modality to evaluate pulmonary blood flow. To couple *DNA* to this targeting agent, *polyethyleneimine* (a polycation that binds *DNA* and enhances transfection) was conjugated to serum *albumin*, and the conjugate was *aggregated* by heating to produce particles of 25-100 microm. The resulting particles bound *plasmid* *DNA* avidly, and when injected i.v. in mice, the particles distributed in the peripheral lung tissue in the alveolar interstitium. Particle-bound luciferase *plasmid* transfected a variety of cell lines in vitro, and after i.v. injection, gene expression was detected exclusively in the lung. Using human growth hormone as the encoded foreign Ag for immunization, i.v. injection of the particle-bound *plasmid* elicited both pulmonary mucosal and systemic immune responses, whereas naked *DNA* injected either i.v. or i.m. elicited only systemic responses. Thus, particle-bound *plasmid* *DNA* may have utility for genetic immunization by intravascular delivery to the lung and potentially to other organs and tissues.

Descriptors: Immunity, Mucosal; *Lung--immunology--IM; *Polyethyleneimine --administration and dosage--AD; *Technetium Tc 99m *Aggregated* Albumin --immunology--IM; *Vaccines, DNA--immunology--IM...; Data; Particle Size; Plasmids--administration and dosage--AD; Plasmids--immunology--IM; Plasmids--pharmacokinetics--PK; Polyethyleneimine--pharmacokinetics--PK; T-Lymphocytes, Cytotoxic--immunology--IM; Technetium Tc 99m *Aggregated* Albumin--administration and dosage--AD; Technetium Tc 99m *Aggregated* Albumin--pharmacokinetics--PK; Transfection--immunology--IM; Tumor Cells, Cultured; Vaccines, DNA--administration and dosage--AD; Vaccines, DNA --pharmacokinetics--PK

Chemical Name: Plasmids; Technetium Tc 99m *Aggregated* Albumin; Vaccines, DNA; Polyethyleneimine; DNA

6/3,K/5 (Item 5 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

10397112 99405134 PMID: 10476219

PEGylated *DNA*/*transferrin*-*PEI* *complexes*: reduced interaction with blood components, extended circulation in blood and potential for systemic gene delivery.

Ogris M; Brunner S; Schuller S; Kircheis R; Wagner E

Institute of Biochemistry, University of Vienna, Austria.

Gene therapy (ENGLAND) Apr 1999, 6 (4) p595-605, ISSN 0969-7128 Journal Code: 9421525

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

PEGylated *DNA*/*transferrin*-*PEI* *complexes*: reduced interaction with blood components, extended circulation in blood and potential for systemic gene delivery.

We investigated the in vitro and in vivo properties of DNA/transferrin-polyethylenimine (800 kDa) *complexes* before and after covalent coupling of poly(ethylene glycol) (PEG). Upon incubation with plasma, the positively charged non-PEGylated DNA *complexes* form *aggregates* . Plasma proteins such as IgM, fibrinogen, fibronectin and complement C3 were found to bind to non-PEGylated DNA *complexes*. At DNA concentrations relevant for in vivo gene delivery a strong aggregation of erythrocytes was also observed. PEGylation of the *complexes* strongly reduces plasma protein binding and erythrocyte aggregation. Furthermore, PEGylated complex size was stabilized and had a reduced surface charge. Prolonged circulation in the blood of the PEGylated *complexes* was also observed when injected intravenously. In tumor bearing mice, application of non-PEGylated *complexes* through the tail vein resulted in reporter gene expression in tail and lung, but severe toxicity was observed in some mice. In contrast, PEGylated *complexes* mediated reporter gene transfer to the tumor without significant toxicity.

6/3,K/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10132578 99129210 PMID: 9930349

The size of *DNA*/*transferrin*-*PEI* *complexes* is an important factor for gene expression in cultured cells.

Ogris M; Steinlein P; Kursa M; Mechtler K; Kircheis R; Wagner E
Institute of Biochemistry, University of Vienna, Austria.
Gene therapy (ENGLAND) Oct 1998, 5 (10) p1425-33, ISSN 0969-7128
Journal Code: 9421525
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

The size of *DNA*/*transferrin*-*PEI* *complexes* is an important factor for gene expression in cultured cells.

Under physiological salt concentration, *plasmid* *DNA* complexed with *transferrin*-conjugated or unmodified polyethylenimine (*PEI*, 800 kDa) forms huge (up to > 1000 nm) *aggregates*, unless the individual components are mixed at a highly positive nitrogen/phosphate (N/P) charge ratio. At low ionic strengths, however, small particles with an...

... in a 10-fold (B16F10 cells) to more than 100-fold (Neuro2A cells, K562 cells) reduced luciferase gene expression efficiency in comparison to the large *complexes* formed in physiological salt solutions. Limited transport of the small particles to the cell surfaces is one possible reason for this effect. Application of the...

... Reduced intracellular release may be another explanation for the decreased transfection efficiency; incubation with chloroquine or incorporation of the endosomolytic peptide INF5 into the small *complexes* enhances gene expression approximately 10-fold. Analysis of gene expression at the cellular level using a green fluorescence *protein* reporter gene and flow cytometry revealed that the differences in overall gene expression largely result from different intensities per expressing cell, while the difference in...
?ds

Set	Items	Description
S1	195	(PLASMID OR DNA) (S) (PEI OR POLYETHYLENEIMINE) (S) (ALBUMIN OR TRANSFERRIN OR PROTEIN)
S2	89	S1 AND (CONJUGATES OR COMPLEXES)

```
 S3          10   S2 AND (ANTIGEN)
 S4           3   RD (unique items)
 S5          16   S2 AND (AGGREGATED OR AGGREGATES)
 S6           6   RD (unique items)
 ?s (plasmid or vector or DNA) (s) (PEI or polylysine or polyethyleneimine or polyamino
 acids or (polycationic (w) polymer))
           159257  PLASMID
           239722  VECTOR
          1938791  DNA
             3035  PEI
             6941  POLYLYSINE
             1857  POLYETHYLENEIMINE
               18  POLYAMINO ACIDS
             2233  POLYCATIONIC
            68883  POLYMER
               38  POLYCATIONIC(W)POLYMER
       S7    1831  (PLASMID OR VECTOR OR DNA) (S) (PEI OR POLYLYSINE OR
                   POLYETHYLENEIMINE OR POLYAMINO ACIDS OR (POLYCATIONIC (W)
                   POLYMER))
 ?s s7 (s) (albumin or transferrin or protein or ligand)
            1831  S7
          220248  ALBUMIN
           53121  TRANSFERRIN
         3300634  PROTEIN
          238492  LIGAND
       S8    580  S7 (S) (ALBUMIN OR TRANSFERRIN OR PROTEIN OR LIGAND)
 ?s s8 and (aggregates or complexes or aggregated)
             580  S8
           57106  AGGREGATES
          295637  COMPLEXES
           31111  AGGREGATED
       S9    248  S8 AND (AGGREGATES OR COMPLEXES OR AGGREGATED)
 ?s s9 and (genetic (w) vaccine)
             248  S9
         1224138  GENETIC
          174169  VACCINE
             107  GENETIC(W)VACCINE
      S10      0  S9 AND (GENETIC (W) VACCINE)
 ?s s9 and (gene (w) delivery)
             248  S9
         2077865  GENE
          313831  DELIVERY
           10506  GENE(W)DELIVERY
      S11     85  S9 AND (GENE (W) DELIVERY)
 ?s s11 and (antigen)
              85  S11
          828966  ANTIGEN
      S12     10  S11 AND (ANTIGEN)
 ?rd
 ...completed examining records
      S13      3  RD (unique items)
 ?t s13/3,k/all

 13/3,K/1     (Item 1 from file: 155)
 DIALOG(R)File 155:MEDLINE(R)

 11164817   21214798   PMID: 11313828
   MHC class II presentation of endogenously expressed antigens by
 transfected dendritic cells.
   Diebold S S; Cotten M; Koch N; Zenke M
   Max-Delbruck-Center for Molecular Medicine, MDC, Berlin, Germany.
   Gene therapy (England)   Mar 2001,  8  (6)  p487-93,  ISSN 0969-7128
 Journal Code: 9421525
   Document type: Journal Article
   Languages: ENGLISH
   Main Citation Owner: NLM
```

Record type: Completed

... I and class II molecules in association with costimulatory molecules, and efficiently activate both cytotoxic T cells and T helper cells. Gene modified DC expressing *antigen* encoding cDNA represent a particularly attractive approach for the immunotherapy of disease. We previously described a *gene* *delivery* system for DC based on receptor-mediated endocytosis of *ligand*/polyethylenimine (*PEI*) *DNA* transfer *complexes* that target cell surface receptors which are abundantly expressed on DC. Employing this *gene* *delivery* system, DC were generated that express chicken ovalbumin (OVA) cDNA as a model *antigen* and introduce *antigen* into the MHC class I presentation pathway. We demonstrate here that modification of OVA cDNA as *transferrin* receptor (TfR) or invariant chain (Ii) fusions effectively generate MHC class II specific immune responses in addition to MHC class I responses. TfR-OVA contains the membrane anchoring region of *transferrin* receptor and represents a membrane-bound form of OVA for access to the MHC class II compartment. Ii-OVA fusions directly target the MHC class II processing pathway. Thus, modification of *antigen* encoding cDNA represents a convenient and effective means to direct antigens to MHC class II presentation and thus to generate T cell help.

13/3,K/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10316864 99315848 PMID: 10383411
Mannose polyethylenimine conjugates for targeted DNA delivery into dendritic cells.
Diebold S S; Kursa M; Wagner E; Cotten M; Zenke M
Max-Delbruck-Center for Molecular Medicine, Robert-Rossle-Str. 10, D-13092 Berlin, Germany.
Journal of biological chemistry (UNITED STATES) Jul 2 1999, 274 (27) p19087-94, ISSN 0021-9258 Journal Code: 2985121R
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Cell surface-bound receptors represent suitable entry sites for *gene* *delivery* into cells by receptor-mediated endocytosis. Here we have taken advantage of the mannose receptor that is highly expressed on *antigen* -presenting dendritic cells for targeted gene transfer by employing mannosylpolyethylenimine (ManPEI) conjugates. Several ManPEI conjugates were synthesized and used for formation of ManPEI/*DNA* transfection *complexes* . Conjugates differed in the linker between mannose and polyethylenimine (*PEI*) and in the size of the *PEI* moiety. We demonstrate that ManPEI transfection is effective in delivering *DNA* into mannose receptor-expressing cells. Uptake of ManPEI/*DNA* *complexes* is receptor-specific, since *DNA* delivery can be competed with mannosylated *albumin* . Additionally, incorporation of adenovirus particles into transfection *complexes* effectively enhances transgene expression. This is particularly important for primary immunocompetent dendritic cells. It is demonstrated here that dendritic cells transfected with ManPEI/*DNA* *complexes* containing adenovirus particles are effective in activating T cells of T cell receptor transgenic mice in an *antigen*-specific fashion.

13/3,K/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10234377 99224851 PMID: 10210145
Efficient *gene* *delivery* into human dendritic cells by adenovirus polyethylenimine and mannose polyethylenimine transfection.
Diebold S S; Lehrmann H; Kursa M; Wagner E; Cotten M; Zenke M
Max-Delbruck-Center for Molecular Medicine, Berlin, Germany.
Human gene therapy (UNITED STATES) Mar 20 1999, 10 (5) p775-86,

ISSN 1043-0342 Journal Code: 9008950
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Efficient *gene* *delivery* into human dendritic cells by adenovirus polyethylenimine and mannose polyethylenimine transfection.

Gene-modified human dendritic cells (DCs) were generated by transfection with adenovirus polyethylenimine *DNA* (Ad/*PEI*/*DNA*) and mannose polyethylenimine *DNA* (ManPEI/*DNA*) *complexes*. Ad/*PEI*/*DNA* *complexes* have *plasmid* *DNA* bound to adenovirus particles by *PEI* and deliver *DNA* into cells via the adenovirus infection route. Such transfection *complexes* yield high transduction levels and sustained expression of luciferase and green fluorescent *protein* reporter genes and were almost as effective as recombinant adenovirus vectors. ManPEI/*DNA* *complexes* rely on uptake by receptor-mediated endocytosis via mannose receptor, which is highly expressed on DCs. While *gene* *delivery* by ManPEI/*DNA* *complexes* was less efficient than by Ad/*PEI* transfection, incorporation of adenovirus particles in ManPEI/*DNA* transfection *complexes* further enhanced transduction efficiencies and transgene expression. We also demonstrate that Ad/*PEI*-transfected DCs are competent in stimulating T cell proliferation in allogeneic and autologous mixed lymphocyte reactions, and in activating T cells from T cell receptor (TCR)-transgenic mice in an *antigen*-specific manner. Thus, the present study establishes the following relative order of transduction efficiencies of viral and nonviral *gene* *delivery* systems for primary human DCs: recombinant adenovirus > Ad/*PEI* = Ad/ManPEI > ManPEI > *PEI*. Ad/*PEI* and ManPEI transfection modes represent particularly versatile transduction systems for DCs, with ManPEI being built up exclusively of synthetic compounds.

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?ds

Set     Items   Description
S1        195   (PLASMID OR DNA) (S) (PEI OR POLYETHYLENEIMINE) (S) (ALBUM-
              IN OR TRANSFERRIN OR PROTEIN)
S2         89   S1 AND (CONJUGATES OR COMPLEXES)
S3         10   S2 AND (ANTIGEN)
S4          3   RD (unique items)
S5         16   S2 AND (AGGREGATED OR AGGREGATES)
S6          6   RD (unique items)
S7       1831   (PLASMID OR VECTOR OR DNA) (S) (PEI OR POLYLYSINE OR POLYE-
              THYLENEIMINE OR POLYAMINO ACIDS OR (POLYCATIONIC (W) POLYMER))
S8        580   S7 (S) (ALBUMIN OR TRANSFERRIN OR PROTEIN OR LIGAND)
S9        248   S8 AND (AGGREGATES OR COMPLEXES OR AGGREGATED)
S10         0   S9 AND (GENETIC (W) VACCINE)
S11        85   S9 AND (GENE (W) DELIVERY)
S12        10   S11 AND (ANTIGEN)
S13         3   RD (unique items)
?s s9 and (ubiquitin or (signal (w) sequence))
            248  S9
          21903  UBIQUITIN
         560636  SIGNAL
        1328521  SEQUENCE
          13822  SIGNAL(W)SEQUENCE
     S14      0  S9 AND (UBIQUITIN OR (SIGNAL (W) SEQUENCE))
?s s11 and (HIV)
             85  S11
         319609  HIV
     S15      6  S11 AND (HIV)
?rd
...completed examining records
     S16      2  RD (unique items)
?t s16/3,k/all

 16/3,K/1     (Item 1 from file: 155)
```

DIALOG(R)File 155:MEDLINE(R)

12623530 21580822 PMID: 11724296

Inhibition of histone-mediated gene transfer in eucaryotic cells by anti-histone IgG.

Hasselmayer O; Demirhan I; Chandra A; Bayer M; Muller R; Chandra P
Gustav-Embden Center of Biological Chemistry, Department of Molecular Biology, Frankfurt University Medical School, Germany.

Anticancer research (Greece) Jul-Aug 2001, 21 (4A) p2377-86, ISSN 0250-7005 Journal Code: 8102988
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

... H4 were found to have the highest transfection efficiency of all the agents tested. In the present study we have analyzed other parameters important for *gene* *delivery* by the histones H3 and H4. We transferred the *HIV*-1 tat gene to Jurkat cells and measured the transactivation of *HIV*-1-LTR by the transactivator *protein*, expressed in Jurkat cells. The expression of CAT as a reporter gene hybridized to LTR was a direct measure of transactivation potential. In order to...
...whether the transfection was only due to the positive ionic character of the histones H3 and H4 we tested other histones (H1 and H2A) and *polylysine* in our system. Under our experimental conditions, neither *polylysine* , nor the histones H1 and H2A were able to promote gene transfer in Jurkat cells. The inability of these reagents to promote gene transfer was not dependent on *DNA* condensation; in EMSA (Electrophoretic Mobility Shift Assay) all these reagents exhibited a strong retardation of *DNA* . In the presence of anti-histone-IgG the transfection potential of histones H3 and H4 was diminished in a concentration - dependent manner. To investigate whether the histone antibodies inhibited the condensation of *DNA* by histones we carried out gel retardation assays (EMSA) in the absence and in the presence of histone antibodies. Anti-histone-IgG had no effect on the retardation of histone-*DNA* *complexes*; on the contrary, retardation was increased. This observation has led us to postulate two models for the possible mechanism by which the histones H3 and...

16/3,K/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10217857 99211168 PMID: 10195264

Histone-mediated transfer and expression of the *HIV*-1 tat gene in Jurkat cells.

Demirhan I; Hasselmayer O; Chandra A; Ehemann M; Chandra P
Gustav-Embden Center of Biological Chemistry, Department of Molecular Biology, Frankfurt University Medical School, Germany.

Journal of human virology (UNITED STATES) Nov-Dec 1998, 1 (7) p430-40, ISSN 1090-9508 Journal Code: 9805755
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Histone-mediated transfer and expression of the *HIV*-1 tat gene in Jurkat cells.

...and Lipofectin have lower transfection efficiency; Lipofectamine has a 2.5-fold better efficiency compared with DEAE-Dextran. We report a novel and highly efficient *DNA* transfer system based on the *DNA*-binding proteins histone 3 and histone 4. We have transferred the *HIV*-1 tat gene and measured the transactivation of *HIV*-1 LTR by the transactivator *protein*, expressed in Jurkat cells. The *HIV*-1 LTR was linked to the CAT gene as a reporter. Compared to DEAE-Dextran-mediated transfection, histone-mediated transfection resulted in a sevenfold higher expression of the CAT gene. The maximum transfection efficiency mediated by histones is

dependent on the relative concentration (*DNA* :histone ratio) and the incubation time. In a gel-retardation assay, an optimal complex formation was observed under the same conditions that allowed the highest transfection efficiency. This ability of histones to increase the delivery and transgenic expression of foreign *DNA* in eukaryotic cells is not simply due to the positive ionic character of the histone proteins. *Polylysine* , histone H1, and histone H2A were unable to mediate gene transfection in our system. Monoclonal antibodies that recognize antigenic determinant present on all five histone...

... were able to neutralize the transfection-enhancing potential of histone 3 and histone 4. However, anti-histone IgG enhanced the retardation of mobility of histone-*DNA* *complexes*. The results of this study allow us to conclude that histones H3 and H4 can catalyze gene transfer and gene expression in eukaryotic cells without any requirement for additional constituents. For this reason, we have termed the new *gene*-*delivery* system as histonefection.

Descriptors: Gene Products, tat--genetics--GE; **HIV*-1--genetics--GE; *Histones--metabolism--ME; *Transfection--methods--MT; Cation Exchange Resins--metabolism--ME; Gene Expression; Gene Products, tat--biosynthesis --BI; Genes, Reporter--genetics--GE; Genetic Vectors--administration and dosage--AD; *HIV* Long Terminal Repeat--genetics--GE; *HIV*-1--chemistry --CH; Jurkat Cells; Lipids--metabolism--ME; Phosphatidylethanolamines --metabolism--ME; Recombinant Proteins--biosynthesis--BI; Time Factors; Trans-Activation (Genetics)--genetics--GE

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?ds

Set     Items   Description
S1        195   (PLASMID OR DNA) (S) (PEI OR POLYETHYLENEIMINE) (S) (ALBUM-
                 IN OR TRANSFERRIN OR PROTEIN)
S2         89   S1 AND (CONJUGATES OR COMPLEXES)
S3         10   S2 AND (ANTIGEN)
S4          3   RD (unique items)
S5         16   S2 AND (AGGREGATED OR AGGREGATES)
S6          6   RD (unique items)
S7       1831   (PLASMID OR VECTOR OR DNA) (S) (PEI OR POLYLYSINE OR POLYE-
                 THYLENEIMINE OR POLYAMINO ACIDS OR (POLYCATIONIC (W) POLYMER))
S8        580   S7 (S) (ALBUMIN OR TRANSFERRIN OR PROTEIN OR LIGAND)
S9        248   S8 AND (AGGREGATES OR COMPLEXES OR AGGREGATED)
S10         0   S9 AND (GENETIC (W) VACCINE)
S11        85   S9 AND (GENE (W) DELIVERY)
S12        10   S11 AND (ANTIGEN)
S13         3   RD (unique items)
S14         0   S9 AND (UBIQUITIN OR (SIGNAL (W) SEQUENCE))
S15         6   S11 AND (HIV)
S16         2   RD (unique items)
?s s9 and (mucosal or subcutaneous or gastrointestinal)
           248  S9
        135779  MUCOSAL
        134973  SUBCUTANEOUS
        325905  GASTROINTESTINAL
     S17    10  S9 AND (MUCOSAL OR SUBCUTANEOUS OR GASTROINTESTINAL)
?rd
...completed examining records
     S18     4  RD (unique items)
?t s18/3,k/all

 18/3,K/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11324766   21374374   PMID: 11459939
  M cell-targeted DNA vaccination.
  Wu Y; Wang X; Csencsits K L; Haddad A; Walters N; Pascual D W
  Veterinary Molecular Biology, Montana State University, Bozeman, MT
59717-3610, USA.
  Proceedings of the National Academy of Sciences of the United States of
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America (United States) Jul 31 2001, 98 (16) p9318-23, ISSN 0027-8424
Journal Code: 7505876
Contract/Grant No.: AI 42673; AI; NIAID; DE 13812; DE; NIDCR; S10 RR11877 ; RR; NCRR
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

DNA immunization, although attractive, is poor for inducing *mucosal* immunity, thus limiting its protective value against most infectious agents. To surmount this shortcoming, we devised a method for *mucosal* transgene vaccination by using an M cell *ligand* to direct the *DNA* vaccine to *mucosal* inductive tissues and the respiratory epithelium. This *ligand*, reovirus *protein* final sigma1, when conjugated to *polylysine* (PL), can bind the apical surface of M cells from nasal-associated lymphoid tissues. Intranasal immunizations with *protein* final sigma1-PL-*DNA* *complexes* produced antigen-specific serum IgG and prolonged *mucosal* IgA, as well as enhanced cell-mediated immunity, made evident by elevated pulmonary cytotoxic T lymphocyte responses. Therefore, targeted transgene vaccination represents an approach for enabling *DNA* vaccination of the mucosa.

18/3,K/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11259307 21286400 PMID: 11389995
Tumor targeting with surface-shielded ligand--polycation DNA *complexes*.

Kircheis R; Blessing T; Brunner S; Wightman L; Wagner E
Boehringer Ingelheim Austria, Dr. Boehringer-Gasse 5-11, A-1121 Vienna, Austria.
Journal of controlled release : official journal of the Controlled Release Society (Netherlands) May 14 2001, 72 (1-3) p165-70, ISSN 0168-3659 Journal Code: 8607908
Document type: Journal Article; Review; Review, Tutorial
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Tumor targeting with surface-shielded ligand--polycation DNA *complexes*.

Incorporation of the receptor binding ligands *transferrin* (Tf) or epidermal growth factor (EGF) into *DNA*/polyethylenimine (*PEI*) *complexes* was found to enhance gene transfer into tumor cell lines in a receptor-dependent manner. In systemic applications, the surface charge of *DNA* *complexes* dominated the in vivo characteristics of gene transfer. Administration of surface-shielded Tf-polycation/*DNA* *complexes* into the tail vein of A/J mice resulted in preferential gene delivery into distantly growing *subcutaneous* Neuro2a tumors. In contrast, application of positively charged *DNA*/*PEI* *complexes* directed gene transfer primarily to the lung. Two alternatives of masking the surface charge of *complexes* were accomplished. In the first case, shielding was obtained by covalently coating of *DNA*/Tf-*PEI* *complexes* with polyethylene glycol (PEG). Alternatively, incorporation of sufficient Tf *protein* into the *DNA* *complexes* resulted in charge shielding even without PEGylation. In the latter case lower-molecular weight polycations (25 kDa *PEI* for Tf-*PEI* *complexes*, or 32 kDa *polylysine* for AVET *complexes*) were used.

18/3,K/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10754932 20302782 PMID: 10843685
Genetic immunization with lung-targeting macroaggregated

polyethyleneimine-albumin conjugates elicits combined systemic and *mucosal* immune responses.

Orson F M; Kinsey B M; Hua P J; Bhogal B S; Densmore C L; Barry M A

Veterans Affairs Medical Center, Baylor College of Medicine, Houston, TX 77030, USA. forson@bcm.tmc.edu

Journal of immunology (Baltimore, Md. : 1950) (UNITED STATES) Jun 15 2000, 164 (12) p6313-21, ISSN 0022-1767 Journal Code: 2985117R

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

Genetic immunization with lung-targeting macroaggregated polyethyleneimine-albumin conjugates elicits combined systemic and *mucosal* immune responses.

Genetic immunization is a novel form of vaccination in which transgenes are delivered into the host to produce the foreign *protein* within host cells. Although systemic immune responses have been relatively easy to induce by genetic immunization, the induction of regional and *mucosal* immunity has often been more challenging. To address the problem of eliciting *mucosal* immunity in the lung, we utilized macroaggregated *albumin* to target *plasmid* *DNA* to the lung. Macroaggregated *albumin* is trapped in the lung after i. v. injection, and it is routinely used in radiolabeled form as an imaging modality to evaluate pulmonary blood flow. To couple *DNA* to this targeting agent, *polyethyleneimine* (a polycation that binds *DNA* and enhances transfection) was conjugated to serum *albumin*, and the conjugate was *aggregated* by heating to produce particles of 25-100 microm. The resulting particles bound *plasmid* *DNA* avidly, and when injected i.v. in mice, the particles distributed in the peripheral lung tissue in the alveolar interstitium. Particle-bound luciferase *plasmid* transfected a variety of cell lines in vitro, and after i.v. injection, gene expression was detected exclusively in the lung. Using human growth hormone as the encoded foreign Ag for immunization, i.v. injection of the particle-bound *plasmid* elicited both pulmonary *mucosal* and systemic immune responses, whereas naked *DNA* injected either i.v. or i.m. elicited only systemic responses. Thus, particle-bound *plasmid* *DNA* may have utility for genetic immunization by intravascular delivery to the lung and potentially to other organs and tissues.

Descriptors: Immunity, *Mucosal*; *Lung--immunology--IM; *Polyethyleneimine--administration and dosage--AD; *Technetium Tc 99m *Aggregated* Albumin --immunology--IM; *Vaccines, DNA--immunology--IM; Amino Acid Sequence; Antibody Formation--genetics--GE; Cell Line; Cytotoxicity, Immunologic --genetics--GE; DNA--administration and dosage--AD; DNA--immunology--IM; DNA--metabolism--ME; Immunity, *Mucosal*--genetics--GE; Lung--metabolism --ME; Lymphocyte Transformation--genetics--GE; Mice; Mice, Inbred BALB C; Molecular Sequence Data; Particle Size; Plasmids--administration and dosage --AD; Plasmids--immunology--IM; Plasmids--pharmacokinetics--PK; Polyethyleneimine--pharmacokinetics--PK; T-Lymphocytes, Cytotoxic--immunology--IM; Technetium Tc 99m *Aggregated* Albumin--administration and dosage--AD; Technetium Tc 99m *Aggregated* Albumin--pharmacokinetics--PK; Transfection --immunology--IM; Tumor Cells, Cultured; Vaccines, DNA--administration and dosage--AD; Vaccines, DNA--pharmacokinetics--PK

Chemical Name: Plasmids; Technetium Tc 99m *Aggregated* Albumin; Vaccines, DNA; Polyethyleneimine; DNA

18/3,K/4 (Item 4 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

10605799 20146298 PMID: 10680017

Gene transfer facilitated by a cellular targeting molecule, reovirus protein sigma1.

Wu Y; Boysun M J; Csencsits K L; Pascual D W

Veterinary Molecular Biology, Montana State University, Bozeman, MT 59717-3610, USA.

Gene therapy (ENGLAND) Jan 2000, 7 (1) p61-9, ISSN 0969-7128

Journal Code: 9421525
Contract/Grant No.: AI42673; AI; NIAID; S10RR11877; RR; NCRR
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

To facilitate eventual genetic vaccination of *mucosal* tissues, a receptor-mediated gene transfer system was devised using the reovirus adhesin, *protein* sigma1. Highly efficient uptake and internalization of *protein* sigma1 *polylysine* (PL) *DNA* *complexes* could be demonstrated by fluorescent microscopy. Successful cellular transfection of rodent and human cell lines was obtained with the recombinant *protein* sigma1 as a PL-*DNA* complex, and could be shown to be receptor-specific. Transfection efficiency was dependent upon the ratio of *DNA* complexed to *protein* sigma1-PL and chloroquine treatment improved transfection efficiency dramatically. To test its ability to bind a *mucosal* inductive tissue, recombinant *protein* sigma1 was specifically bound to the nasal-associated lymphoid tissues (NALT). Thus, recombinant *protein* sigma1-PL-*DNA* conjugates can efficiently bind and transfect cells that express the receptor for *protein* sigma1. Gene Therapy (2000) 7, 61-69.
?ds

Set	Items	Description
S1	195	(PLASMID OR DNA) (S) (PEI OR POLYETHYLENEIMINE) (S) (ALBUMIN OR TRANSFERRIN OR PROTEIN)
S2	89	S1 AND (CONJUGATES OR COMPLEXES)
S3	10	S2 AND (ANTIGEN)
S4	3	RD (unique items)
S5	16	S2 AND (AGGREGATED OR AGGREGATES)
S6	6	RD (unique items)
S7	1831	(PLASMID OR VECTOR OR DNA) (S) (PEI OR POLYLYSINE OR POLYETHYLENEIMINE OR POLYAMINO ACIDS OR (POLYCATIONIC (W) POLYMER))
S8	580	S7 (S) (ALBUMIN OR TRANSFERRIN OR PROTEIN OR LIGAND)
S9	248	S8 AND (AGGREGATES OR COMPLEXES OR AGGREGATED)
S10	0	S9 AND (GENETIC (W) VACCINE)
S11	85	S9 AND (GENE (W) DELIVERY)
S12	10	S11 AND (ANTIGEN)
S13	3	RD (unique items)
S14	0	S9 AND (UBIQUITIN OR (SIGNAL (W) SEQUENCE))
S15	6	S11 AND (HIV)
S16	2	RD (unique items)
S17	10	S9 AND (MUCOSAL OR SUBCUTANEOUS OR GASTROINTESTINAL)
S18	4	RD (unique items)

?s s9 and (mucosal or systemic)

	248	S9
	135779	MUCOSAL
	418254	SYSTEMIC
S19	34	S9 AND (MUCOSAL OR SYSTEMIC)

?rd
...completed examining records

S20	12	RD (unique items)

?s s20 not s18

	12	S20
	4	S18
S21	8	S20 NOT S18

?t s21/3,k/all

21/3,K/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

13393996 22131122 PMID: 12136428
Tumor-targeted gene delivery of tumor necrosis factor-alpha induces tumor necrosis and tumor regression without *systemic* toxicity.
Kircheis Ralf; Ostermann Elinborg; Wolschek Markus F; Lichtenberger Cornelia; Magin-Lachmann Christine; Wightman Lionel; Kursa Malgorzata;

Wagner Ernst
Boehringer Ingelheim Austria, Vienna, Austria.
Cancer gene therapy (England) Aug 2002, 9 (8) p673-80, ISSN 0929-1903 Journal Code: 9432230
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: In Process

Tumor-targeted gene delivery of tumor necrosis factor-alpha induces tumor necrosis and tumor regression without *systemic* toxicity.
We have recently developed surface-shielded *transferrin* -polyethylenimine (Tf-*PEI*)/*DNA* delivery systems that target reporter gene expression to distant tumors after *systemic* application. In the present study, we used surface-shielded Tf-*PEI*/*DNA* *complexes* for delivering the gene for a highly potent cytokine, tumor necrosis factor-alpha (TNFalpha). TNFalpha is known for its ability to induce hemorrhagic tumor necrosis and tumor regression. However, the therapeutic application of TNFalpha is hampered by its high *systemic* toxicity dictating the need to target TNFalpha activity to the tumor. *Systemic* application of surface-shielded Tf-*PEI* *complexes* with the TNFalpha gene resulted in preferential expression of TNFalpha in the tumor without detectable TNFalpha serum levels, in contrast to the application of nontargeted *complexes* . Tumor-targeted TNFalpha gene delivery induced pronounced hemorrhagic tumor necrosis and inhibition of tumor growth in three murine tumor models of different tissue origins, Neuro2a neuroblastoma, MethA fibrosarcoma, and M-3 melanoma, with complete tumor regressions observed in the MethA model. No *systemic* TNF-related toxicity was observed due to the localization of the TNFalpha activity to the tumor. Targeted gene therapy may be an attractive strategy applicable...

21/3,K/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11306665 21352555 PMID: 11459457
Different strategies for formation of pegylated EGF-conjugated PEI/DNA *complexes* for targeted gene delivery.
Blessing T; Kursa M; Holzhauser R; Kircheis R; Wagner E
Institute of Medical Biochemistry, University of Vienna, Dr. Bohrgasse 9/3, A-1030 Vienna and Boehringer Ingelheim Austria, Dr. Boehringer Gasse 5-11, A-1121 Vienna, Austria. blessing@pharma.univ-montp1.fr
Bioconjugate chemistry (United States) Jul-Aug 2001, 12 (4) p529-37, ISSN 1043-1802 Journal Code: 9010319
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Different strategies for formation of pegylated EGF-conjugated PEI/DNA *complexes* for targeted gene delivery.
With the aim of generating gene delivery systems for tumor targeting, we have synthesized a conjugate consisting of polyethylenimine (*PEI*) covalently modified with epidermal growth factor (EGF) peptides. Transfection efficiency of the conjugate was evaluated and compared to native *PEI* in three tumor cell lines: KB epidermoid carcinoma cells, CMT-93 rectum carcinoma cells, and Renca-EGFR renal carcinoma cells. Depending on the tumor cell line, incorporation of EGF resulted in an up to 300-fold increased transfection efficiency. This *ligand* -mediated enhancement and competition with free EGF strongly suggested uptake of the *complexes* through the EGF receptor-mediated endocytosis pathway. Shielded particles being crucial for *systemic* gene delivery, we studied the effect of covalent surface modification of EGF-*PEI*/*DNA* *complexes* with a poly(ethylene glycol) (PEG) derivative. An alternative way for the formation of PEGylated EGF-containing *complexes* was also evaluated where EGF was projected away from *PEI*/*DNA* core *complexes* through a PEG

linker. Both strategies led to shielded particles still able to efficiently transfect tumor cells in a receptor-dependent fashion. These PEGylated EGF-containing *complexes* were 10- to 100-fold more efficient than PEGylated *complexes* without EGF.

21/3,K/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

11250857 21295020 PMID: 11402299
Polyethylenimine/DNA *complexes* shielded by transferrin target gene expression to tumors after *systemic* application.
Kircheis R; Wightman L; Schreiber A; Robitza B; Rossler V; Kursa M; Wagner E
Boehringer Ingelheim Austria, Dr Boehringer Gasse 5-11, A-1121 Vienna, Austria.
Gene therapy (England) Jan 2001, 8 (1) p28-40, ISSN 0969-7128 Journal Code: 9421525
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Polyethylenimine/DNA *complexes* shielded by transferrin target gene expression to tumors after *systemic* application.
Systemic application of positively charged polycation/*DNA* *complexes* has been shown to result in predominant gene expression in the lungs. Targeting gene expression to other sites, eg distant tumors, is hampered by nonspecific interactions largely due to the positive surface charge of transfection *complexes* . In the present study we show that the positive surface charge of *PEI* (25 kDa branched or 22 kDa linear)/*DNA* *complexes* can be efficiently shielded by covalently incorporating *transferrin* at sufficiently high densities in the complex, resulting in a dramatic decrease in nonspecific interactions, eg with erythrocytes, and decreased gene expression in the lung. *Systemic* application of *transferrin*-shielded *PEI*/*DNA* *complexes* into A/J mice bearing subcutaneously growing Neuro2a tumors via the tail vein resulted in preferential (100- to 500-fold higher) luciferase reporter gene expression in distant tumors as compared with the major organs including the lungs. Tumor targeting is also demonstrated by *DNA* uptake and beta-galactosidase gene expression in tumor cells. Assessing *DNA* distribution following *systemic* application significant amounts of *DNA* were found in the liver and tumor. However, in the liver, *DNA* was mainly taken up by Kupffer cells and degraded without significant transgene expression. In the tumor, *DNA* was associated mainly with tumor cells and frequently found near structures which resemble primitive blood vessels.

21/3,K/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10966052 20534468 PMID: 11083497
Biodistribution and transgene expression with nonviral cationic vector/DNA *complexes* in the lungs.
Bragonzi A; Dina G; Villa A; Calori G; Biffi A; Bordignon C; Assael B M; Conese M
Institute for Experimental Treatment of Cystic Fibrosis, San Raffaele Scientific Institute, Milano, Italy.
Gene therapy (ENGLAND) Oct 2000, 7 (20) p1753-60, ISSN 0969-7128 Journal Code: 9421525
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Biodistribution and transgene expression with nonviral cationic

vector/DNA *complexes* in the lungs.

Biodistribution of nonviral cationic *vector*/*DNA* *complexes* was studied after *systemic* or intratracheal administration to the lungs and correlated with transgene expression. Intravenous injection in C57Bl/6 mice gave maximal and significant luciferase expression in the lungs with the cationic polymer *PEI* 22K/*DNA* *complexes* at the highest ratios of positive/negative charges versus *DNA* alone. While DOTAP/*DNA* *complexes* with high charge ratio determined lower but still significant luciferase activity versus uncomplexed *DNA*, GL-67A and *PEI* 25K mediated negligible luciferase expression. Labelled *PEI* 22K and DOTAP *complexes* were evenly distributed in the alveolar region, where GFP expression was revealed, while *PEI* 25K and GL-67A *complexes* were not detected, suggesting a different interaction of these *complexes* with the plasma membrane of endothelial cells. Following an intratracheal injection, the highest and significant levels of transfection were obtained with slightly positive *PEI* *complexes* as compared with *DNA* alone, whereas cationic lipid-based vectors, DOTAP and GL-67A, gave not significant luciferase activity. Both types of polyplexes gave similar levels of lung luciferase expression by targeting different airway cell populations. *PEI* 25K *complexes* determined high levels of GFP in the bronchial cells, confirming confocal data on fluorescent *complexes* internalization. *PEI* 22K *complexes* gave mainly high GFP signal in the distal tract of the bronchial tree, where tagged *complexes* were recovered. Fluorescent lipid *complexes* were found in *aggregates* in the lumen of bronchi totally (DOTAP) or partially (GL-67A) co-localizing with surfactant *protein* A. Results indicated that cationic polymers could overcome the surfactant barrier which inhibited airway cell transfection mediated by cationic lipids.

21/3,K/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10731174 20267143 PMID: 10809146

***Systemic* linear polyethylenimine (L-PEI)-mediated gene delivery in the mouse.**

Zou S M; Erbacher P; Remy J S; Behr J P

Laboratoire de Chimie Genetique, Faculte de Pharmacie de Strasbourg, France.

journal of gene medicine (ENGLAND) Mar-Apr 2000, 2 (2) p128-34, ISSN 1099-498X Journal Code: 9815764

Document type: Journal Article

Languages: ENGLISH

Main Citation Owner: NLM

Record type: Completed

***Systemic* linear polyethylenimine (L-PEI)-mediated gene delivery in the mouse.**

BACKGROUND: Several nonviral vectors including linear polyethylenimine (L-*PEI*) confer a pronounced lung tropism to *plasmid* *DNA* when injected into the mouse tail vein in a nonionic solution. METHODS: and results We have optimized this route by injecting 50 microg *DNA* with excess L-*PEI* (*PEI* nitrogen/*DNA* phosphate = 10) in a large volume of 5% glucose (0.4 ml). In these conditions, 1-5% of lung cells were transfected (corresponding to 2 ng luciferase/mg *protein*), the other organs remaining essentially refractory to transfection (1-10 pg luciferase/mg *protein*). beta-Galactosidase histochemistry confirmed alveolar cells, including pneumocytes, to be the main target, thus leading to the puzzling observation that the lung microvasculature must be permeable to cationic L-*PEI*/*DNA* particles of ca 60 nm. A smaller injected volume, premixing of the *complexes* with autologous mouse serum, as well as removal of excess free L-*PEI* , all severely decreased transgene expression in the lung. Arterial or portal vein delivery did not increase transgene expression in other organs. CONCLUSIONS: These observations suggest that effective lung transfection primarily depends on the injection conditions: the large nonionic glucose bolus prevents aggregation as well as mixing of the

cationic *complexes* and excess free L-*PEI* with blood. This may favour vascular leakage in the region where the vasculature is dense and fragile, i.e. around the lung alveoli. Cationic particles...

21/3,K/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10658666 20203062 PMID: 10738575
Polycation-based DNA *complexes* for tumor-targeted gene delivery in vivo.
Kircheis R; Schuller S; Brunner S; Ogris M; Heider K H; Zauner W; Wagner E
Boehringer Ingelheim Austria, Vienna, Austria.
journal of gene medicine (ENGLAND) Mar-Apr 1999, 1 (2) p111-20, ISSN 1099-498X Journal Code: 9815764
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Polycation-based DNA *complexes* for tumor-targeted gene delivery in vivo.
... fluids and extracellular matrix, and binding to a broad variety of non-target cell types. METHODS: Polycation-based vectors, including adenovirus-enhanced transferrinfection (AVET) and *transferrin* -polyethylenimine (Tf-*PEI*), were tested for gene delivery into subcutaneously growing tumors after local and *systemic* application. *DNA* biodistribution and reporter gene expression was measured in the major organs and in the tumor. RESULTS: Gene transfer after intratumoral application was 10-100 fold more efficient with Tf-*PEI*/*DNA* or AVET *complexes* in comparison to naked *DNA* . Targeted gene delivery into subcutaneously growing tumors after *systemic* application was achieved using electroneutral AVET *complexes* and sterically stabilized PEGylated Tf-*PEI*/*DNA* *complexes* , whereas application of positively charged polycation/*DNA* *complexes* resulted in predominant gene expression in the lungs and was associated by considerable toxicity. CONCLUSION: For *systemic* application, the physical and colloidal parameters of the transfection *complexes* , such as particle size, stability, and surface charge, determine *DNA* biodistribution, toxicity, and transfection efficacy. By controlling these parameters, *DNA* biodistribution and gene expression can be targeted to different organs.

21/3,K/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10495208 20031559 PMID: 10563778
Multicomponent DNA carrier with a vesicular stomatitis virus G-peptide greatly enhances liver-targeted gene expression in mice.
Schuster M J; Wu G Y; Walton C M; Wu C H
Department of Medicine, Division of Gastroenterology-Hepatology, University of Connecticut School of Medicine, Farmington, Connecticut 06030, USA.
Bioconjugate chemistry (UNITED STATES) Nov-Dec 1999, 10 (6) p1075-83 , ISSN 1043-1802 Journal Code: 9010319
Contract/Grant No.: DK-42182; DK; NIDDK
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

Genes can be targeted to hepatocytes in vitro and in vivo by the use of asialoorosomucoid-*polylysine* conjugates. After *systemic* application, this nonviral *vector* is recognized by highly selective asialoglycoprotein (AsGP) receptors on the sinusoidal liver cell membrane and is taken up via

receptor-mediated endocytosis. As most of the *DNA* is rapidly transferred to lysosomes where it is degraded, transfection efficiency is low and gene expression transient. To address this problem, we incorporated a pH-dependent synthetic hemolytic peptide derived of the G-*protein* of Vesicular Stomatitis Virus (VSV) into the gene transfer system, to increase endosomal escape of internalized *DNA*. The multicomponent carrier binds *DNA* in a nondamaging way, is still recognized by the AsGP receptor, and is targeted to the liver in vivo. Injection of *DNA* *complexes* containing a luciferase marker gene resulted in luciferase expression of 29 000 pg/g liver which corresponded to an increase of a factor of 10(3) overexpression after injection of *DNA* *complexes* without endosomolytic peptide. Furthermore, the amount of intact transgene within isolated liver cell nuclei was increased by a factor of 10(1)-10(2) by the use of the multicomponent carriers. These results demonstrate that incorporation of a hemolytic peptide into a nonviral *vector* can greatly increase gene expression while retaining cell type targetability in vivo.

21/3,K/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

10397112 99405134 PMID: 10476219
PEGylated *DNA*/*transferrin*-*PEI* *complexes*: reduced interaction with blood components, extended circulation in blood and potential for *systemic* gene delivery.
Ogris M; Brunner S; Schuller S; Kircheis R; Wagner E
Institute of Biochemistry, University of Vienna, Austria.
Gene therapy (ENGLAND) Apr 1999, 6 (4) p595-605, ISSN 0969-7128
Journal Code: 9421525
Document type: Journal Article
Languages: ENGLISH
Main Citation Owner: NLM
Record type: Completed

PEGylated *DNA*/*transferrin*-*PEI* *complexes*: reduced interaction with blood components, extended circulation in blood and potential for *systemic* gene delivery.
We investigated the in vitro and in vivo properties of DNA/transferrin-polyethylenimine (800 kDa) *complexes* before and after covalent coupling of poly(ethylene glycol) (PEG). Upon incubation with plasma, the positively charged non-PEGylated DNA *complexes* form *aggregates* . Plasma proteins such as IgM, fibrinogen, fibronectin and complement C3 were found to bind to non-PEGylated DNA *complexes*. At DNA concentrations relevant for in vivo gene delivery a strong aggregation of erythrocytes was also observed. PEGylation of the *complexes* strongly reduces plasma protein binding and erythrocyte aggregation. Furthermore, PEGylated complex size was stabilized and had a reduced surface charge. Prolonged circulation in the blood of the PEGylated *complexes* was also observed when injected intravenously. In tumor bearing mice, application of non-PEGylated *complexes* through the tail vein resulted in reporter gene expression in tail and lung, but severe toxicity was observed in some mice. In contrast, PEGylated *complexes* mediated reporter gene transfer to the tumor without significant toxicity.
?ds

Set	Items	Description
S1	195	(PLASMID OR DNA) (S) (PEI OR POLYETHYLENEIMINE) (S) (ALBUMIN OR TRANSFERRIN OR PROTEIN)
S2	89	S1 AND (CONJUGATES OR COMPLEXES)
S3	10	S2 AND (ANTIGEN)
S4	3	RD (unique items)
S5	16	S2 AND (AGGREGATED OR AGGREGATES)
S6	6	RD (unique items)
S7	1831	(PLASMID OR VECTOR OR DNA) (S) (PEI OR POLYLYSINE OR POLYETHYLENEIMINE OR POLYAMINO ACIDS OR (POLYCATIONIC (W) POLYMER))
S8	580	S7 (S) (ALBUMIN OR TRANSFERRIN OR PROTEIN OR LIGAND)

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S9          248   S8 AND (AGGREGATES OR COMPLEXES OR AGGREGATED)
S10           0   S9 AND (GENETIC (W) VACCINE)
S11          85   S9 AND (GENE (W) DELIVERY)
S12          10   S11 AND (ANTIGEN)
S13           3   RD (unique items)
S14           0   S9 AND (UBIQUITIN OR (SIGNAL (W) SEQUENCE))
S15           6   S11 AND (HIV)
S16           2   RD (unique items)
S17          10   S9 AND (MUCOSAL OR SUBCUTANEOUS OR GASTROINTESTINAL)
S18           4   RD (unique items)
S19          34   S9 AND (MUCOSAL OR SYSTEMIC)
S20          12   RD (unique items)
S21           8   S20 NOT S18
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         1126732  REVIEW?
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     $5.85  TELNET
    $32.50  Estimated cost this search
    $33.37  Estimated total session cost   5.212 DialUnits


### Status: Signed Off. (27 minutes)
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